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Carl W. Walter, M. D.
Ruth B. Kundsin, Sc. D.

Peter Bent Brigham Hospital

Bionomics of the Staphylococcus in the Nasal Carrier

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Principal Investigators: Carl W. Walter, M.D., Ruth B. Kundsin, Sc. D.

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1. Substances that inhibit the growth of colonies of bacteria were demonstrated. These diffuse through an agar impregnated bacterial filter.

2. Gram-negative bacteria are most inhibitory to other bacteria, but show little interaction with other species of gram-negative bacteria.

3. Strains of Staphylococcus aureus show little inhibition against other species, but exhibit mutual inhibition against other strains of staphylococci.

4. Ninety-four per cent of 32 medical students carried Mycoplasma species. Forty-one per cent carried gram-negative flora in their nostrils; 31 per cent in their throats.

5. Initially 50 per cent and 34 per cent were persistant nasal and throat carriers respectively of S. aureus. After exposure to the hospital environment, these figures shifted to 65 and 63 per cent - a 22 per cent increase in rate of carriage.

6. Statistical analysis of the data indicate possible in vivo evidence for the antibacterial interaction of organisms.

7. The importance of gram-negative infection as a cause of morbidity and mortality in patients with life endangering illness was demonstrated. Of 42 patients in this category, 31 had major infection associated with refractory hypotension complicating a surgical problem. Twenty had positive blood cultures; 19 were due to gram-negative bacteria. Death in gram negative bacteremic shock occurred in 65 per cent.

8. The microcirculation in the hamster cheek pouch was quantitated.

9. The pharmacodynamics of the microvasculature in the hamster cheek pouch was explored. The reaction of the vessels to vasoactive substances, including gram-negative endotoxin, was measured.

10. A method for the bioassay of gram-negative toxin in human plasma was explored.

11. Cells suggestive of human platelets have been grown in vitro.

Key words: Staphylococcal carriers
Gram-negative carriers
Mycoplasma carriers
Antibacterial substances
Microvasculature of hamster cheek pouch
Pharmacodynamics of circulation in cheek pouch
Assay of gram-negative toxin
Platelets

PROGRESS REPORT

Since moving into the renovated laboratory during May 1964. the various research programs have progressed in a more orderly and timely manner. Personnel have been relieved of the frustration of crowding and gain satisfaction from their work. The laboratory has been able to accommodate limited clinical problems of special interest. These contacts have been stimulating, and also provided opportunities to obtain material that would otherwise not have occurred.

Work on the staphylococcal aspects of the project has lagged somewhat because gram-positive wound infections have become sparse and disseminating carriers essential for the study have not been identified to replace those previously under study whose careers took them elsewhere.

Because gram-negative infections have been of major import among critically ill surgical patients, special emphasis was placed in this area. A research fellow, a fourth-year medical student and a competent bacteriologist were assigned to study this problem. The major share of their support was from resources of the department of surgery.

The major activities of the laboratory are supported by allied contracts DA-49-193-MD-2455, "Bionomics of the Staphylococcus in the Nasal Carrier", and DA-49-193-MD-2494, "The Prevalence and

Importance of PPLO and L Forms". Personnel from both contracts have been used to support progress on either project as was demanded by clinical opportunity, work load or program need.

This report is organized in three parts to reflect areas of special interest.

1. Staphylococcus
   - 1.1. Bionomics of the staphylococcus
   - 1.2. Indigenous flora of the respiratory tract of medical students
2. Gram-negative infections
   - 2.1. Septic shock and terminal sepsis in critically ill patients
   - 2.2. Microvasculature of the cheek pouch in the hamster
   - 2.3. Pharmacodynamics of the microvasculature in the cheek pouch in the hamster
   - 2.4. Bioassay of plasma for vasoconstrictive substances and gram-negative endotoxin
3. Growth of human platelets <u>in vitro</u>

1.1. <u>Bionomics of the Staphylococcus</u> - The purpose of this study is to describe the relationship between the Staphylococcus aureus and its habitat in the human nasopharynx. The goal is to determine what influences colonization and abets dissemination of the staphylococcus.

<u>In vitro interaction of colonies of bacteria</u> - A series of studies were made on the interacting effect on growth of

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colonies of different species and strains of bacteria developing in paired proximity. Initially the technic described by Rosebury, Zeitinger, and Mogab[1] was used. Inoculation of nutrient agar by overlapping drops of a suspension of bacteria permitted observation of contrasting degrees of growth of the paired colonies.

Both inhibiting and stimulating interactions were noted. To determine whether the interaction was due to a diffusible substance rather than to contiguity effects of deprivation of nutrients, attempts were made to separate the growing colonies by the interposition of a membrane filter.

Some effort was expended in developing a technic for tidal perfusion of the paired colonies when each was grown on a membrane separated by 2 mm. from the other membrane in back-to-back tandem position. Much work remains to be done on this technic before significant results can be obtained.

Promising results were more readily achieved by pairing inoculations on either side of a millipore membrane (pore size 0.45) which had been impregnated with nutrient agar. The membrane was suspended in a millipore field monitor for inoculation.

Bacteria studied and their source

Bacillus cereus - settling plate - fallout from burn patient

Escherichia coli - isolated from chest wall of burn patient

Klebsiella species - unknown

Pseudomonas aeruginosa - trachea - autopsy burn patient

Staphylococcus epidermidis - nasal culture

Staphylococcus aureus 502A Eichenwald 7/53/77/42B/83B/ Group III

Staphylococcus aureus - skin of burned patient Leavitt 81/82

Staphylococcus aureus - throat culture - UC-18

Staphylococcus aureus - healthy nasal carrier 3B/3C/55/71 Group II

Staphylococcus aureus - healthy nasal carrier 52/52A/79/80 Group I

Staphylococcus aureus - stock propagating strain for phage 71

These organisms were tested against themselves and each other by placing one drop of an overnight broth culture on opposing sides of a millipore filter. Controls were inoculated on the appropriate side of each filter so normal growth of the drop of bacteria on the filter could be observed at a distance from competing colonies. The second or opposing drop was placed at intervals 1, 2, 3, 4, 5, 6, hours; therefore, the first drop always had a longer growth period than the second drop. After incubation, growth status of the colonies of bacteria was observed. In some instances the filters were stained with malachite green and dried. The filters were read for the amount of inhibition caused by each colony against the paired colony on the opposite side of the membrane. This was quantitated in the basis of a scale 0 (no inhibition) to 5 (maximal inhibition).

Results

1.1.1. Varied intervals of head start in growth seemed

to show no effect on the phenomenon of inhibition. However further study must be done to bear this out.

1.1.2. Data were analyzed by scoring the results of the individual experiments. Tables I and II show the cumulative scores.

Inhibitory effect of test organisms against paired opponents in order of highest inhibitory power to lowest.

Klebsiella species - most inhibitory to other species showed little inhibition against itself

Pseudomonas aeruginosa

Escherichia coli

Staphylococcus aureus 71

Bacillus cereus

Staphylococcus aureus 502A

Staphylococcus aureus UC-18

Staphylococcus aureus 81/82

Staphylococcus aureus 52/52A/79/80

Staphylococcus aureus 3B/3C/55/71

Staphylococcus epidermidis - least inhibitory to other species

The inhibitory effect of paired opponent on the test organism reciprocal of aforementioned series:

Staphylococcus aureus 52/52A/79/80 most inhibited itself

Staphylococcus aureus 3B/3C/55/71

Staphylococcus aureus 502A

Staphylococcus aureus 81/82

Staphylococcus aureus 71

Staphylococcus aureus UC-18

Staphylococcus epidermidis

Bacillus cereus

Escherichia coli

Klebsiella species

Pseudomonas aeruginosa - least inhibited itself

Conclusions

1.1.3. Gram-negative bacteria are most inhibitory to other bacteria. Gram-negative bacteria showed no mutual inhibition.

1.1.4. Strains of S. aureus were least inhibitory to other species and exhibited most mutual inhibition (most benign versus gram-negatives and bacteriostatic against other strains of staphylococci).

1.1.5. B. cereus - close to gram-negative bacteria in being inhibitory and in being inhibited.

1.1.6. S. epidermidis - least inhibitory - not inhibited much by itself.

1.1.7. S. aureus - all strains act similarly and can be grouped together.

1.1.8. The inhibitory substance diffused through an agar impregnated membrane having a pore size of 0.45 μ. Whether

this is a toxin or antibiotic is a matter of conjecture.

1.1.9. These studies may explain the clinical observation in burn patients where the initial infection by gram-positive organisms is followed in succession by the gram-negatives in order: E. coli, Klebsiella, Pseudomonas. The most inhibitory species ultimately becoming dominant.

1.1.10. The mutual inhibition among the strains of S. aureus explains the finding of pure isolates of these bacteria in contrast to the mixed cultures usually encountered in gram-negative infections. In the latter, mutual inhibition has trivial influence.

Studies are in progress in which filtrates of month-old cultures are being diffused into colonies of bacteria growing on agar impregnated membrane in the attempt to quantitate the inhibitory effect and discover its nature.

1.2. <u>The indigenous flora of the respiratory tract in medical students</u>

The dynamics of the carrier status of a group of 32 second-year medical students is being determined. The study began while the students were chiefly engaged in preclinical laboratory and lecture exercises. As they experienced increasing exposure to the hospital environment, it was anticipated that a shift in flora would be detected that would differentiate the interaction of the indigenous bacterial flora in each student with those encountered in the hospital.

Nasal and throat cultures were taken at weekly intervals beginning in January 1965. In collaboration with Contract No. DA-49-193-MD-2494, Mycoplasma species were also isolated. It was a surprise to discover that 94 per cent of the students carried Mycoplasma species.

No less surprising was the finding that 41 per cent carried gram-negative flora in their nostrils at least once; 25 per cent persistantly; 31 per cent carried these organisms in their throats.

Initially, 50 per cent and 34 per cent were persistant nasal and persistant throat carriers of Staphylococcus aureus respectively. With exposure to the hospital environment, these figures shifted to 65 and 63 per cent, a 22 per cent increase. It is noteworthy that 25 per cent of the students have never carried S. aureus.

Preliminary analysis of the data indicates possible in vivo antibacterial interaction of organisms.

The significance of these data awaits phage typing and serologic studies.

Several undeterminable bacteria were recovered that may be important factors in determining colonization of the nasopharynx. These may be comparable to a strain of B. cereus that was recovered from a severely burned patient about a year ago. While this organism flourished in the wound, skin, and respiratory tract, the expected sequence of invasion by S. aureus was delayed 10 days.

These studies suggest that the subsidence of staphylococcal

disease in the hospital may have resulted from the dominance of the gram-negative bacteria in the environment.

As a result of the increasing prevalence of gram-negative infections, the colony of staphylococcus typing bacteriophage has been maintained at high titer. This activity occupies one technician. During the year 2488 specimens were typed.

2. Gram-negative infection - Dr. Adrian Litton M.D.C.H.B. of Glasgow spent a year in the laboratory. He directed his efforts toward the study of the bacteriology of patients with life-endangering illness. Because hypotension with collapse of the peripheral circulation was the dominant problem, he chose to study the circulation in the microvasculature of the hamster cheek pouch. Both the clinical and laboratory projects ultimately involved gram-negative endotoxin. A technic for the bioassay of gram-negative endotoxin evolved.

2.1. Septic shock and terminal sepsis in critically ill patients

Forty-two patients with life-endangering illness were studied in the Intensive Care Unit. This work was reported at the National Academy of Science Workship, Washington, D.C., September 11, 1964, in a paper entitled, "Septic Shock and Terminal Sepsis in Critically Ill Surgical Patients", Adrian Litton, Carl W. Walter, Ruth B. Kundsin, and will appear in the Proceedings of that conference.(2) Reprints will be submitted as soon as they are available. Thirty-one patients had major infection, associated with refractory hypotension complicating a surgical

problem. Twenty had positive blood cultures. In one instance S. aureus UC-18 was isolated. The other 19 were due to gram-negative organisms. Bacterial cultures from respiratory or urinary tracts, skin and wounds grew the same organism, and after 24-48 hours colonization, the dominant organism was frequently found in the blood. Oliguria from acute renal tubular necrosis, coagulation defects, ulceration of the alimentary tract with hemorrhage, bronchopneumonia, hypoxia and metabolic acidosis were the additional complications. In spite of energetic treatment, death in gram-negative bacteremic shock occurred in 65 per cent.

2.2. The microvasculature of the cheek pouch in the hamster

The microcirculation in the cheek pouch of the hamster became of interest as a means of understanding the peripheral circulatory changes in patients with hypotension resulting from gram-negative sepsis. Dr. Litton acquired the technics from Dr. Herbert Berman in the Biology Research Center at Boston University. The work was presented in part at the Annual Meeting of the American Physiological Society at Brown University, Providence, Rhode Island, September 9, 1964[3]. Reprints of an abstract published in the Proceedings of that meeting accompany.

Method - The microvasculature was quantitated in the cheek pouch of the living anesthetized hamster. Camera lucida drawings and photographs were made of distribution of the vessels. Diameters and lengths of vessels were measured with a calibrated ocular

micrometer. Endothelial surface areas, blood volume and total cross-sectional areas were calculated from the different types of blood vessels. For each size of blood vessel, the mean of 25 readings was recorded. Magnifications varied from 40X to 1200X depending on the vessels being measured. The hamsters were anesthetized with 9 mgms. nembutol/100 G intra-peritoneally. When required for study of the smaller vessels, single membrane preparations were made by cutting a window in the buccal membrane of the everted cheek pouch.

Results - There were usually 3-4 main arteries entering the cheek pouch and a similar number of major veins leaving it.

Table III shows the mean for the number and dimensions of the main orders of arteries and veins in the cheek pouch. Branches from the main arteries formed the main arterial arcade in the cheek pouch. These anastomoses occurred throughout the cheek pouch, and ensured an even distribution to the terminal arterioles and the capillary bed. The mean diameter of the arterioles forming the arcades was 28.6 micra. The areas varied widely but an average was 3 mm. x 1.5 mm. Smaller arterial anastomoses occasionally occurred, but this was infrequent.

No arterio-venous communications were seen.

The anastomosing channels on the venous side were much more numerous than on the arterial side. This occurred at all levels of confluence from the second order of post-capillary up to the largest veins. This rich anastomosis, in addition to maintaining

a uniform pressure gradient and flow pattern, also provided a large sump for the storage of blood. In the cheek pouch, there were usually four orders of arteries, capillaries and five orders of veins. There vessels have been designated arbitrarily as:

A 4 Main artery

A 3 Artery

A 2 Small artery

A 1 Terminal arteriole

C Capillary

V 5 Main vein

V 4 Vein

V 3 Small vein

V 2 Venule

V 1 Post-capillary venule

Table IV shows the lengths and diameters of these vessels.

The length, total cross-sectional area of the various arteries, capillaries and veins is demonstrated in Table V.

It can be seen that there is a gradual increase in cross-sectional area down to the small arteries. A three-fold increase then occurs to the terminal arteriole and a further two-fold increase to the capillary bed. It is in these terminal two orders of arteries that the maximum pressure decrements must occur.

In the present study, the capillary bed did not possess the

largest cross-sectional area. Further small, but significant, increases occurred in the first two orders of veins and it was not until the third order of veins that the area was smaller than that of the capillary bed (90.2 per cent). Further along the venous channels showed a gradual decrease in cross-sectional area.

The capillary vessels formed a uniform network throughout the cheek pouch with arterial supply and venous drainage joining the network.

The area between adjoining capillaries had a mean value of 113 micra. In the cheek pouch under resting conditions only one third of the capillary vessels were open at any one time. The average diameter of the capillary was 4.84 $\pm$ 0.6 micra.

The size of capillary vessel observed in the living state was smaller than the erythrocytes which became deformed during their transit of the capillary. This inconguity of size may allow maximum apposition of red cell membrane and vascular endothelium - perhaps important in blood and tissue gaseous exchange.

The blood volume capacity on the venous side was much greater than on the arterial. Three fourths of the blood in the micro-circulation is on the venous side.

As endothelial surface area controls the diffusion to and from the tissue, calculation of the relative proportion of

endothelium in the various vessels was made. This is shown in Table VI. Although only 2.2 per cent of the total blood volume was in the capillaries, those vessels accounted for 14.0 per cent of the total endothelial area. The ratio of per cent endothelium and per cent volume is recorded in Table VII.

Discussion and Conclusion - The hamster cheek pouch has a rich vascular network. There are no hair follicles or glandular structures in the cheek pouch and the variations in the metabolic requirements are not as varied as glandular or muscular tissue. There are no arterio-venous shunts present in the cheek pouch.

There is a linear increase in cross-sectional area in the last two orders of arteries up to the capillaries. The first and second order of post-capillary venules have the largest cross-sectional area in the microcirculation. Anastomoses on the arterial side ensure an even distribution of pressure to the capillary bed. The capillary vessels have a diameter less than that of the undistorted erythrocytes. Three quarters of the blood in the microcirculation is on the venous side.

2.3. The pharmacodynamics of the microvasculature in the cheek pouch of the hamster

Knowledge of the action of vasoactive substances on the microvasculature became of paramount importance in the assay of endotoxin or biologic fluids. The drugs likely to be used on patients with hypotension were studied to permit sorting out the pharmacologic from the pathophysiologic responses of the

microcirculation. The material is being prepared for publication.

2.3.1. Method - The substances were diluted to the same volume (0.05 ml.) with sterile Ringer's Solution. Injection was made into the areola tissue between the epidermal membranes of the pouch with a No. 30 needle and tuberculin syringe. The injection caused a slight blister to rise in the buccal membrane at a site carefully chosen so that several arteries and veins remained in focus. When the solution was applied to the surface of the pouch, no change occurred, apparently because of the lack of diffusion through epidermal layer. When a single membrane preparation was made, the solution to be tested was dropped on the exposed areola tissue and then covered with a glass slide. The same effects were observed as following the injection between the membranes of the cheek pouch. One advantage of injection was that subsequent moistening of the pouch with Ringer's Solution did not dilute the substance being tested.

Anesthesia profoundly effects the sympathetic vasoconstrictive tone. All experiments were carried out under the same conditions (9 mgms. Nembutol/100 gm. body weight) and compared with the injection of Ringer's Solution as a control in each instance.

2.3.2. Results - Control experiments with Ringer's Solution are tabulated in Table VIII.

There was no change in the cross-sectional area of either artery or vein. Control injections were made in each experiment to permit comparison of the observations and determinations of the change in cross-section area.

Histamine - Injection of 0.05 µg. and 10 µg. of histamine increased the cross-section of the arteries (36 per cent) while decreasing the veins (26 per cent) thereby decreasing the peripheral arterial resistance while constricting the venous bog. See Tables IX and X.

Catecholamines - epinephrine - see Table XI caused constriction of arteries to 2.5 per cent of control values and veins to 37 per cent. The constriction persisted until relieved at 30 minutes by the injection of phenoxybenzamine or phentolamine.

norepinephrine - See Table XII - constricted the arteries to 2 per cent and the veins to less than 24 per cent of the control values until relieved after 30 minutes by the injection of phenoxybenzamine and phentolamine.

Vasoactive dilator substances - Local injection of phenoxybenzamine and phentolamine are inhibitors of the alpha cell receptor action of the catecholamines. Given by themselves, the former drugs had no action, but given after the vasopressors (epinephrine and norepinephrine) they seemed to neutralize the vasoconstrictive action.

Acetylcholine - caused equal dilatation of arteries and veins as shown in Table XIII.

Angiotensin II - had a marked vasoconstrictor effect to 7 per cent of control values that did not persist. It is presumably degraded by angiotensinase effect of tissue fluids. A continuous infusion to prolong the effect of angiotensin vasoconstrictor was required. See Table XIV.

Serotonin - has a more prolonged arterial vasoconstrictor effect to 20-40 per cent of control values, but contrary to the other published reports, the cheek pouch veins did not show vasoconstriction as with norepinephrine. See Table XV.

Lactic Acid - caused vasodilation. It was not discovered whether this was due to lactic acid itself or merely was a function of the lower pH. See Table XVI. No other acids were tried.

Cortisone - had no direct vasoactive effect - See Table XVII.

Isoproterenal - This substance is reported to be a pure beta cell receptor-activator and as such should cause dilatation of both arteries and veins. In all the experiments, the veins dilated promptly but the arteries uniformly constricted - whether this was a result of the improperly proportioned pharmacological dose was not elicited. This interesting substance was the mirror image of histamine. The arteries constricted while the veins dilated; whereas

with histamine, arteries dilated and veins constricted. These two substances were the only ones that produced opposite effects on the arteries and veins.

Blood flow through a tissue or organ is a function of the vasoactivity of the artery and vein. Where the arteries constricted relatively more than the veins, stasis was observed in the post-capillary venules, encouraging transcapillary exchange from tissues to capillary lumen. When venous constriction was associated with arterial dilatation, the capillaries became distended and ultimately all of them opened (see action of histamine). Transcapillary exudation into the tissues occurred and extracellular tissue edema was observed. At the end of one hour the fine focus on the vessels was difficult because of this edema.

Results

Intravenous administration - The drugs listed were also given intravenously into the femoral vein and the same effects were observed as in a local injection. Endotoxin was the sole exception.

Endotoxin - injected locally gave a response similar to that of histamine release. The arteries dilated to 125 per cent of the control values while the vein contracted to 10 per cent. Histamine was the only substance which caused contraction of the capacitance vessels (veins) and

dilatation of the resistance vessels (arteries).

The histamine-like response of endotoxin injected locally is shown in Table XVIII. Whether this is due to a direct action of endotoxin on the endothelial cells of the minute vessels or is mediated through the pericytes and mast cells in the neighborhood of the vessels was not discovered.

From previous reports and papers, endotoxin is cleared from the blood by the reticuloendothelial system. If the reticuloendothelial system is blocked (e.g. by thorotrast) the lethality of endotoxin is increased. After intravenous injection of endotoxin, there is marked vasoconstriction and hypotension. See Table XIX. This catecholamine-like response appears to be secondary to peripheral pooling of blood caused by intravenous injection of endotoxin.

Platelet emboli - Direct microscopy of the cheek pouch before and after the intravenous injection of the endotoxin showed the aggregation of platelets in the post-capillary venules 3 to 6 minutes after injection. The aggregates increased in size and fragments broke off and were carried into the larger veins as platelet emboli. Only occasionally did platelet aggregates completely plug a vessel with the cessation of flow due more or less to red thrombus formation. The showers of platelet emboli were marked 3 to 15 minutes after intravenous injection of endotoxin. After 30 minutes

most of the emboli had disappeared.

In three hamsters platelet counts were done to demonstrate the depletion of circulating platelets by the action of endotoxin.

Blood was collected by direct cardiac puncture and transferred to a "Unopette" (B.D.) and the count was done on a phase contrast microscope. See Table XX.

2.4. Bioassay of plasma for vasoconstrictive substances and gram-negative endotoxin

Several experiments were done to test the vasoactivity of the plasma especially in patients with shock associated with infection.

2.4.1. Normal human serum caused vasoconstriction to about 60 per cent of the cross-section area of the controls. This is probably due to serotonin release for disintegrating platelets.

2.4.2. Normal human heparinized plasma caused only slight vasoconstriction; maximal observed was 80 per cent of the controls. The blood was collected and spun immediately. Only the top layer of plasma taken - care being made not to include any of the buffy layer - 0.05 ml. of undiluted plasma was injected into the cheek pouch.

2.4.3. Heparinized plasma from patients in the hospital, some postoperative and some without operation but not in shock - slight vasoconstriction as seen with heparinized

plasmas from normal persons.

2.4.4. Patients in "septic shock" - plasma collected at same time as blood cultures that were positive for gram-negative bacteria caused vasoconstriction effects to 20-50 per cent of control values. When vasodilator was added to cheek pouch 30 minutes after the toxic plasma was injected, the vasoconstrictor effect was removed.

2.4.5. Heparinized plasma from burned patients - Three patients with greater than 50 per cent body surface burns were tested at 3-5 weeks after burns. They were NOT hypotensive at the time. The plasma of two out of three of patients had most pronounced vasoconstrictor effect.

2.4.6. Plasma of one patient in septic shock with a septicemia due to Klebsiella-Aerobacter and on a norepinephrine drip (2 amps. in 1 bottle Ringer's Solution). The patient's blood pressure was maintained with difficulty at 80 mm. Hg. systolic, and there was profound coldness and cyanosis of extremities. The plasma was markedly vasoconstrictive. (The effect that the levophed was having on the patient's vessels was shown also in the cheek pouch vessels.) See Table XXI.

When the plasma was added to the cheek pouch, profound arterial vasoconstriction occurred. Although there was little change in the cross-sectional area of the veins,

dramatic stasis of blood with red cell aggregation was observed. After the injection of phentolamine after 30 minutes, the arterial spasm was removed and blood flow returned rapidly to normal.

Discussion - This work is a promising approach to the evaluation of pharmacologic-etiology of disturbances of the circulation in critically ill patients. Unfortunately it was discontinued at a crucial point in the development by Dr. Litton's return to Glasgow.

3. Growth of human platelets in vitro - Aliquots of platelet concentrates of human plasma have been inoculated into culture media of various compositions. The platelets were harvested by centrifuging freshly drawn ACD blood at 1400 g. for 3 minutes. The plasma was expressed and centrifuged at 1400 g. for an additional 30 minutes. During incubation at 37°C., a spherical translucent mass grew. Smears of this tissue stained with Wright's stain showed spherical or ovoid bodies of varying size suggestive of platelets.

There is an extensive report in the literature by C. Xalabardar of Barcelona, Spain. The Intriguing Biology of Blood Platelets (in vitro cultures)[4] This was published in Publicaciones de Instituto Antituberalis, Vol. XIII, p. 5-94, 1959.

Table I

Rating Values for Each Experiment

	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	Cumulative rating
B. cereus	4	5	7	7.5	6	6.5	36
E. coli	3	1.5	2	2.5	7.5	6.5	23
Klebsiella	2	1.5	2	2.5	1.5	1.5	11
Pseudonomas	1	3	4.5	1	1.5	8	19
S. epidermidis	11	11	9	11	11	11	64
502A	8	8	2	5	4.5	10	37.5
81	7	6	6	7.5	3	9	38.5
UC-18	6	7	8	6	7.5	3.5	38
3B	10	10	10	10	9	5	54
52	9	9	11	9	10	3.5	51.5
71	5	4	4.5	4	4.5	1.5	23.5

Table II

Rating Values for Each Experiment

	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	Cumulative rating
B. cereus	7	8	8	8	9	9	49
E. coli	8.5	9	9	9	8	8	51.5
Klebsiella	10	10.5	10	10.5	10	10	61
Pseudomonas	11	10.5	11	10.5	11	11	65
S epidermidis	5	3.5	5	7	3	7	30.5
502A	5	3.5	4	3.5	4.5	3.5	24
81	5	6	7	3.5	6	1.5	29
UC-18	8.5	5	3	5	7	6	34.5
3B	2	1.5	2	2	1.5	1.5	10.5
52	1	1.5	1	1	1.5	3.5	9.5
71	3	7	6	6	4.5	5	31.5

Table III

Major Arterial and Venous Vessels in Cheek Pouch

	Main Arteries (A_4)	Anastomosing Arteries (A_3)	Veins (V_4)	Largest Veins (V_5)
Number	3.4	28.9	28.8	3.9
Length μ	9,061	4,590	4,170	11,940
Diameter μ	74.6	28.6	75.7	184.0
Circumference μ	234	90	238	578
Area μ^2	4,371	542	4,501	26,590
Length x No. of Vessels μ	30,807	132,651	120,096	46,566
Total X-sectional Area μ^2	14,861	18,566	129,620	103,701
Endothelial Area in Pouch ($\mu^2 \times 10^6$) (millions)	7.22	11.92	28.56	26.92
Blood Volume per Pouch ($\mu^3 \times 10^6$)	134.7	85.2	540.5	1,238.2

Units of length, area, and volume are in microns (μ, μ^2, and μ^3).

All numbers are averaged from a minimum of 6 pouches.

Table IV

Lengths and Diameters of Minute Vessels

Mean Values (± Standard Error)

	Vessel	Length (mm.)	Diameter (u)
A 4	Main artery	9.1 ± 1.2	74.6 ± 3.6
A 3	Artery	4.6 ± 0.33	28.6 ± 1.2
A 2	Small artery	5.1 ± 0.31	19.0 ± 1.3
A 1	Terminal arteriole	1.55± 0.14	11.72± 0.47
C	Capillary	0.29± 0.0094	4.84± 0.12
V 1	Post-cap. venule	0.309±0.04	9.13± 0.49
V 2	Venule	1.64± 0.22	24.3 ± 1.5
V 3	Small vein	2.5 ± 0.31	35.5 ± 1.9
V 4	Vein	4.17± 0.71	75.7 ± 5.3
V 5	Main vein	11.94± 1.5	184.0 ± 5.0

Table V

Microvasculature of Pouch: Branches (A) and Tributaries (V)

	Length	Diam.	Circ.	Area	Brahches	No. of Vessels	Length x No.	X-sect. area
A_4 Main Artery	9,061	74.6	234.4	4,371	8.5	3.4	30.807	14,861
A_3 Anast. Artery	4,590	28.6	89.9	642	3.4	28.9	132,651	18 566
A_2 Small Artery	5,100	19.0	59.7	284	7.9	99	501,330	27,871
A_1 Terminal Arteriole	1,547	11.7	36.8	108	12.4	777	1,201,400	83,485
C Capillary	290	4.8	15.2	18	-	9,630	2,792,642	177,188
V_1 Post-cap. Vein	309	9.1	28.7	65	3.2	3,009	929,874	197,019
V_2 Venule	1,642	24.3	76.3	464	6.9	436	716,076	202,250
V_3 Small Vein	2,500	35.5	111.5	990	2.7	162	403,750	159,852
V_4 Vein	4,170	75.7	237.8	4,500	5.6	28	120,096	129,620
V_5 Main Vein	11,940	184.0	578.1	26,590	7.3	3.9	46,566	103,701

Table VI

Comparison of Volume and Endothelium *

Vessel	Per Cent of Total Volume	Per Cent of Total Endothelium
Artery	21.5	30.6
Capillary	2.2	14.0
Vein	76.3	55.4

* with V_5 taken as a cone

Table VII

	% Endothelium	% Volume	Endothelium/Volume
Main Artery	2.4	5.8	0.4
Artery	3.9	3.8	1.0
Small artery	9.8	6.3	1.6
Terminal arteriole	14.5	5.6	2.6
Capillary	14.0	2.2	6.4
1st venule	8.8	2.7	3.3
Venule	18.0	14.6	1.2
Small vein	14.8	17.5	0.8
Vein	9.4	23.5	0.4
Main Vein (as cone)	4.4	17.9	0.2

Table VIII

Control Experiments

A) Injection of 0.05 ml. Ringer's Solution into Cheek Pouch

% Control cross-section area ($\Delta \pi R^2$)		Time in Minutes after Injection				
		3 min.	6 min.	15 min.	30 min.	60 min.
Exp. #1	A	82.3	88.3	100.4	84.0	84.0
	V	87.5	93.3	95.9	97.6	100.6
Exp. #2	A	104.3	103.5	94.8	98.9	94.9
	V	98.7	97.1	98.0	100.9	97.3
Exp. #3	A	90.3	96.3	97.8	94.8	-
	V	95.7	101.1	107.5	97.2	-
Exp. #4	A	98.0	97.8	104.2	105.2	108.1
	V	100.7	100.8	95.9	95.8	104.2
Exp. #5	A	98.0	95.4	87.7	95.4	93.1
	V	98.9	96.7	94.8	96.7	100.6
Mean (Arteries)		94.6 (±7.6)	96.3(±5.0)	97.0(±5.2)	95.7(±6.9)	95.0(±8.6)
Mean (Veins)		96.3 (±4.7)	97.8(±2.9)	98.4(±4.6)	97.6(±1.7)	100.7(±2.5)

B) Observation of Cheek Pouch and no Injections

Exp. #1	A	98.5	107.1	102.0	101.6	91.5
	V	97.1	100.3	101.1	91.8	97.6
Exp. #2	A	99.2	97.9	104.2	105.2	108.1
	V	100.7	100.8	95.9	95.8	104.2
Exp. #3	A	101.6	96.4	101.2	99.8	97.7
	V	98.7	96.3	93.9	93.9	98.7
Mean (Arteries)		99.8(±1.4)	100.5(±5.8)	102.5(±1.6)	102.2(±2.2)	99.1(±6.8)
Mean (Arteries)		98.8(±1.4)	99.1(±2.0)	97.0(±3.0)	93.8(±1.6)	100.2(±2.9)

Table IX

Action of Histamine on Microcirculation

1. Local injection of 10 µg. histamine base in 0.05 ml. Ringer's solution

% Control cross-section area		3 min.	6 min.	15 min.	30 min.	60 min.
Exp. #1	A	80.0	114.2	125.9	138.6	129.5
	V	87.3	74.2	78.2	74.4	75.4
Exp. #2	A	90.3	49.0	119.2	134.3	140.2
	V	86.3	73.8	75.4	70.7	76.3
Exp. #3	A	83.4	130.0	138.3	132.6	108.9
	V	74.2	70.7	82.1	70.7	86.6
Exp.#4	A	80.1	122.1	129.0	125.6	126.9
	V	96.5	79.5	78.5	85.4	92.3
Exp. #5	A	79.9	123.5	121.1	117.7	112.7
	V	90.8	82.2	77.6	84.1	87.?
Exp. #6	A	143.2	153.1	136.9	110.3	104 9
	V	91.4	86.0	79.2	87.1	82.5
Mean (arteries) S.D.		92.8(±12.0)	115.3(±32.5)	128.4(±7.2)	126.5(±9.1)	120.1(±119)
Mean (veins)		87.8(±6.8)	77.7(±5.4)	78.5(±2)	78.4(±6.9)	83.5(±6.1)

Table X

Action of Histamine on Microcirculation

2. Local injection of 5 micrograms histamine base in 0.05 ml. Ringer's solution.

% Control cross-section area		3 min.	6 min.	15 min.	30 min.	60 min.
Exp. # 1	A	87.4	121.2	121.2	136.5	147.2
	V	89.8	79.1	82.9	85.0	89.0
Exp. #2	A	80.4	107.5	117.6	120.3	125.3
	V	82.9	74.7	67.1	75.3	74.6
Exp. #3	A	80.0	120.0	131.4	146.8	138.1
	V	84.8	73.9	73.1	67.2	76.4
Exp. #4	A	86.6	-	96.4	126.8	123.7
	V	62.4	-	74.3	88.5	91.8
Exp. #5	A	80.1	88.6	123.6	133.7	147.7
	V	69.1	67.2	90.3	93.4	97.3
Exp. #6	A	51.1	80.2	145.4	145.0	-
	V	79.4	74.2	82.0	86.0	-
Mean (arteries)	S.D.	77.6(±12.3)	103.5(±16.4)	122.6(±11.6)	134.9(±9.5)	136.4(±10.3)
Mean (veins)	S.D.	78.1(±9.4)	73.8(±3.8)	78.3(±7.6)	82.6(±8.8)	85.8(±8.9)

Table XI

Action of Catecholamines on Microcirculation

1. Injection 1 µg. epinephrine followed in 30 min. by injection of 5 µg. Phenoxybenzamine (Dibenzyline)

% Control cross-section area		3 min.	6 min.	15 min.	30 min.	3 min.	6 min.	15 min.	30 min.
Exp. #1	A	6.4	5.4	2.7	2.7	22.3	31.6	34.2	36.0
	V	61.0	52.7	41.9	33.0	40.9	45.8	49.3	47.9
Exp. #2	A	4.9	4.7	5.4	6.2	17.6	21.4	21.1	34.6
	V	75.9	69.2	65.2	62.7	79.1	81.5	66.3	63.9
Exp. #3	A	9.1	8.0	5.9	5.1	24.5	54.0	95.3	95.4
	V	25.0	25.0	21.4	17.3	30.4	45.3	58.2	82.0
Mean (arteries)		6.8	6.0	4.7	4.7	21.5	35.7	50.2	55.3
Mean (veins)		54.0	49.0	42.8	37.7	50.1	57.6	57.9	64.6

2. Injection 1 µg. epinephrine followed in 30 min. by injection 5 µg. Phentolamine (Regitive)

Exp. #1	A	12.8	5.1	4.7	5.1	42.2	102.9	106.2	114.5
	V	52.4	33.9	29.4	23.2	48.1	85.8	85.6	96.2
Exp. #2	A	15.4	5.9	4.6	4.6	50.6	77.1	102.0	99.9
	V	73.8	57.3	45.9	46.2	65.1	89.1	92.7	91.0
Exp. #3	A	6.7	3.7	2.9	1.5	34.4	78.4	88.9	100.4
	V	60.4	43.5	36.1	26.5	46.8	61.1	68.4	79.6
Mean (arteries)		11.6	4.9	4.1	3.7	42.4	86.1	99.0	104.9
Mean (veins)		62.2	44.9	37.1	32.0	53.3	78.7	82.2	88.9

Table XII

Action of Catecholamines on Microcirculation

3. Injection 1 µg. norepinephrine and in 30 min. injection 5 µg. Phenoxybenzamine (Dibenxyline)

% Control cross-section area		3 min.	6 min.	15 min.	30 min.	3 min.	6 min.	15 min.	30 min.
Exp. #1	A	5.5	4.2	15.7	15.7	82.1	109.1	109.4	102.1
	V	72.2	49.4	64.0	57.8	80.1	92.6	92.8	99.4
Exp. #2	A	1.3	1.6	2.6	2.9	42.0	94.1	103.1	101.8
	V	83.6	77.4	53.3	47.7	69.6	86.4	95.5	97.0
Exp. #3	A	2.4	1.3	1.5	5.0	54.6	79.1	96.0	101 6
		34.6	24.7	8.9	6.5	12.9	20.5	61.9	74.2
Mean (arteries)		3.1	2.4	6.6	7.9	59.6	94.1	102.8	107.8
Mean (veins)		63.5	50.5	42.1	37.3	54.2	66.5	83.4	90.2

4. Injection 1 µg. norepinephrine and in 30 min. injection 2 µg. Phentolamine (Regitive)

Exp. #1	A	5.5	1.6	1.5	0.6	31.6	45.9	59.6	71.5
	V	10.8	6.3	4.1	2.3	21.3	38.6	50.9	58.7
Exp. #2	A	17.5	4.8	2.5	1.7	30.1	69.3	96.2	98.8
	V	51.8	40.6	28.6	24.0	51.9	78.7	86.8	83.9
Exp. #3	A	3.2	1.2	1.9	1.2	48.4	106.6	111.4	111.4
	V	60.9	56.2	49.6	46.0	54.6	62.5	62.5	86.8
Mean (arteries)		8.7	2.5	2.0	1.2	36.7	73.9	89.1	93.9
Mean (veins)		41.2	34.4	27.4	24.1	42.6	59.9	73.6	76.5

Table XIII

Action of Acetylcholine on Microcirculation

Injection of 1 µg. Acetylcholine in 0.05 ml. Ringer's solution into cheek pouch

% Control cross-section area ($\Delta \pi R^2$)		Time in minutes after injection				
		3 min.	6 min.	15 min.	30 min.	60 min.
Exp. # 1	A	118.8	128.0	107.7	109.7	108.5
	V	98.2	92.3	91.6	93.0	96.5
Exp. #2	A	112.7	127.1	132.7	142.8	114.8
	V	135.6	159.2	148.2	156.3	134.6
Exp. #3	A	110.7	118.1	123.8	97.5	94.9
	V	102.6	147.6	153.2	147.6	146.4
Exp. #4	A	93.5	115.5	111.8	105.8	91.0
	V	103.8	105.7	114.2	109.8	98.2
Exp. #5	A	112.0	140.3	128.8	142.4	129.1
	V	108.3	117.7	110.9	126.1	117.7
Exp. #6	A	132.7	138.9	128.4	128.1	116.9
	V	129.6	131.0	114.8	109.8	111.6
Mean (Arteries)		113.4(±11.7)	128.0(±9.4)	122.2(±9.2)	121.1(±17.8)	109.2(± 13.2
Mean (Veins)		113.0(±14.3)	125.6(±23.1)	122.2(±21.8)	123.8(±22.2)	117.5(± 18.0

Table XIV

Action of Angiotensin II on Microcirculation

A) 25 µgs. Angiotensin II in 0.05 ml. Ringer's solution

% Control cross-section area ($\Delta\pi r^2$)		3 min.	6 min.	15 min.	30 min.	60 min.
Exp. #1	A	4.8	3.3	11.3	93.7	93.6
	V	68.6	69.4	90.3	91.6	90.2
Exp. #2	A	4.2	16.9	20.7	92.9	105.4
	V	96.0	86.6	82.5	87.8	92.7
Exp. #3	A	12.8	62.3	74.0	88.1	80.1
	V	96.0	94.2	95.3	95.5	99.5
Exp. #4	A	4.6	11.8	61.6	86.4	91.7
	V	90.0	89.5	90.5	95.4	97.1
Mean (arteries)		6.6(±3.6)	23.6(±23.0)	41.9(±26.6)	90.3(±3.1)	92.7(±9.0)
Mean (veins)		87.7(±11.2)	84.9(±9.4)	89.7(±9.2)	92.6(±3.2)	94.9(±3.6)

B) 5 µgs. Angiotensin II

Exp. #1	A	5.4	16.1	31.3	27.7	37.9
	V	79.1	73.3	80.0	74.5	84.7
Exp. #2	A	84.7	83.4	74.5	56.1	52.5
	V	93.1	93.7	91.0	93.2	86.7
Exp. #3	A	26.6	30.9	55.4	79.0	90.3
	V	94.4	96.0	90.9	96.9	100.9
Exp. #4	A	13.7	19.9	54.3	81.2	108.2
	V	96.2	95.5	90.1	93.0	104.2
Mean (arteries)		32.6(±31.0)	37.6(±26.8)	53.9(±15.3)	61.0(±21.5)	72.2(±28.3)
Mean (veins)		90.7(±6.8)	89.6(±9.5)	88.0(±4.7)	89.4(±8.8)	94.1(±8.5)

Table XV

Action of Serotonin on Microcirculation

Local injection 5 µg. Serotonin in Ringer's solution into cheek pouch

% Control cross-section area ($\Delta\pi R^2$)		3 min.	6 min.	15 min.	30 min.	60 min.
Exp. #1	A	10.8	14.1	15.7	19.1	15.1
	V	88.2	88.2	87.9	95.9	99.7
Exp. #2	A	17.8	32.6	32.9	28.6	38.6
	V	97.7	100.2	90.9	86.1	80.3
Exp. #3	A	70.4	31.1	60.4	45.4	44.1
	V	96.4	99.2	100.5	99.8	79.5
Exp. #4	A	4.6	8.5	22.3	21.8	21.7
	V	95.9	90.1	87.0	89.4	85.7
Exp. #5	A	48.1	19.7	64.6	45.5	49.4
	V	99.7	100.7	96.0	98.7	93.8
Exp. #6	A	19.3	9.1	32.1	29.2	73.7
	V	86.1	84.4	95.3	87.7	89.4
Mean (artery) ± SD		28.5(±23.2)	19.2(±9.8)	38.0(±18.7)	31.6(±10.1)	40.4(±19.2)
Mean (veins) ± SD		94.0(±5.0)	93.8(±6.5)	92.9(±5.0)	92.9(±5.4)	88.1(±7.2)

Table XVI

Action of Lactic Acid on the Microcirculation

Injection 100 µg. Lactic Acid in 0.05 ml. Ringer's solution

%Control cross-section area ($\Delta\pi R^2$)		3 min.	6 min.	15 min.	30 min.	60 min.
Exp. #1	A	113.0	127.9	140.0	127.9	106.9
	V	121.2	129.8	127.4	130.1	119.3
Exp. #2	A	89.5	111.4	131.5	119.0	115.3
	V	118.5	118.1	127.2	111.7	100.8
Exp. #3	A	122.3	134.9	140.9	156.1	133.3
	V	115.2	129.8	138.7	128.2	114.4
Mean (arteries)		108.3(±13.8)	124.7(±8.0)	137.5(±4.2)	134.3(±15.8)	118.5(± 11.0)
Mean (veins)		118.3(±2.5)	125.9(±5.5)	131.1(±5.4)	123.3(±8.3)	111.5(±7.8)

Table XVII

Action of Cortisone

Injection of 50 µg. Cortisone Acetate

% Control cross-section area ($\Delta \pi R^2$)		3 min.	6 min.	15 min.	30 min.	60 min.
Exp. #1	A	104.5	100.7	90.2	79.5	80.0
	V	94.6	89.4	90.1	88.4	94.7
Exp. #2	A	98.4	101.6	94.4	92.8	98.3
	V	92.9	90.1	92.9	95.9	99.7
Exp. #3	A	103.3	94.5	103.3	99.8	105.3
	V	99.9	104.1	104.1	110.9	98.1
Mean (arteries)		102.1(±2.6)	98.9(±3.2)	96.0(±6.1)	90.7(±8.4)	94.5(±10.6)
Mean (veins)		95.8(±2.9)	94.5(±6.7)	95.7(±6.0)	98.4(±9.3)	97.5(±2.1)

Table XVIII

Action of E. coli Endotoxin on Microcirculation

A) Local action of 100 µg. E. coli Lipopolysaccharide (026:B6) (Difco) on hamster cheek pouch in 0.05 ml. Ringer's Solution

% Control cross-section area ($\Delta\pi r^2$)		3 min.	6 min.	15 min.	30 min.	60 min.
Exp. #1	A	106.2	115.0	118.3	122.3	120.8
	V	97.8	99.4	92.3	93.8	92.6
Exp. #2	A	113.5	123.0	124.6	127.4	117.5
	V	98.2	92.4	96.6	96.7	96.2
Exp. #3	A	139.4	143.5	146.6	149.4	154.7
	V	94.4	84.8	91.6	94.2	97.0
Exp. #4	A	89.4	112.0	129.2	135.9	141.8
	V	95.5	95.8	86.6	87.5	91.8
Exp. #5	A	109.5	138.9	118.9	110.7	104.1
	V	83.6	72.7	89.3	89.0	94.7
Exp. #6	A	79.1	107.8	107.8	115.6	107.8
	V	97.5	91.7	84.0	91.9	97.5
Mean (arteries) ±SD		106.2 (±19.2)	123.4 (±13.5)	124.2 (±12.0)	126.9 (±13.9)	124.4 (± 18.0)
Mean (Veins) ± SD		94.5 (±5.1)	89.5 (±8.7)	90.1 (±4.0)	92.2 (±2.9)	95.0 (± 2.1)

Table XVIII

B) 500 µg. (injected in 0.05 ml. Ringer's Solution into cheek pouch) — mean results (3 experiments) of control cross-section area

% Control cross-section area ($\Delta\pi r^2$)		3 min.	6 min.	15 min.	30 min.	60 min.
Exp. #1	Arteries	134.5(±23)	166.8(±16.4)	176.1(±0.3)	152.5(±13.2)	133.1(±17.8)
	Veins	85.0(±14)	84.2(±10.6)	74.6(±12)	80.1(±20.5)	84.3(±9.8)
Exp. #2	Crude extract from Difco (0.05 ml. injection) mean results (3 experiments)					
	Arteries	93.0	104.1	119.4	133.4	114.9
	Veins	85.8	82.2	76.9	81.4	85.1
Exp. #3	Crude extract (filtrate on 1/52 old centrifuged culture of E. coli from burn patient M.C. mean results (3 experiments)					
	Arteries	116.3	133.3	176.1	155.2	173.3
	Veins	83.6	75.5	83.4	67.1	74.7

Table XIX

Action of E. coli Endotoxin on Microcirculation

Intravenous injection of 2.0 mgms.endotoxin in 0.5 ml. Ringer's Solution into femeral vein and cheek pouch - observed

(2.0 mgms.026:B.6 Lipopolysaccharide of E. coli is > LD_{100} for hamsters)

% Control cross-section area ($A\pi R^2$)		3 min.	6 min.	15 min.	30 min.	60 min.
Exp. #1	A	88.7	53.6	44.3	57.4	60.9
	V	98.1	90.1	106.4	131.4	117.1
Exp. #2	A	92.5	20.0	28.9	26.0	32.0
	V	96.0	97.3	91.3	96.1	104.7
Exp. #3	A	74.6	72.9	38.9	56.7	76.6
	V	76.2	74.9	52.1	62.6	69.4
Exp. #4	A	65.3	48.1	45.2	37.0	-
	V	95.8	88.0	79.9	77.0	-
Mean (arteries)		80.3(±11.8)	48.7(±19.2)	39.3(±6.5)	44.3(±13.8)	56.5(±18.5)
Mean (veins)		91.5(±8.9)	87.6(±8.1)	82.4(±19.9)	91.8(±25.9)	97.1(±20.0)

Table XX

	Before Endotoxin	30 minutes after 2 mgms. lipopoly-saccharide i.v. (Endotoxin)
Results:		
(1)	744.000	17,000
	767.000	14,000
(2)	742,000	116,000
	766,000	134,000
(3)	782,000	196,000
	753,000	184,000

Table XXI

Phentolamine 10 ug. into cheek pouch ↓

$(\Delta \pi R)^{\frac{1}{2}}$	3 min.	6 min.	15 min.	30 min.	3 min.	6 min.	15 min.	30 min.
Arteries	5.1%	5.6	3.7	10.7	53.4	79.0	78.1	68.0
Veins	100 %	103.3	99.7	92.6	87.4	93.5	98.3	91.8

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THE VASCULAR PATTERN IN THE HAMSTER CHEEK POUCH: A QUANTITATIVE APPROACH. A. Litton, H.J.Berman and G. McCormick. Biol. Res. Ctr., Boston Univ., and Peter Bent Brigham Hosp., Boston, Mass.

The microvasculature has been quantitated in the distal third of the cheek pouch of the living anesthetized hamster, Mesocricetus auratus. Camera lucida drawings and photographs were made of the general and detailed distribution of the vessels. Diameters and lengths of vessels were measured with a calibrated ocular micrometer. Cross-sectional areas, blood volume, and endothelial surface area were calculated for the different types of blood vessels. Magnification varied from 40X to 1200X. Anastomosing arterial arcades maintained an even pressure distribution to the terminal arterioles. No arterial venous anastomoses were noted. The capillaries formed a continuous basketlike network. The small veins formed finer systems of anastomoses than the arterial arcades. Measurements of total cross-sectional areas of the different types of blood vessels in 10 hamsters disclosed a linear relationship from the arterial arcades to capillaries. An approximate three-fold increase was observed from the small artery originating from the arcade to the terminal arteriole, and a similar increase from the terminal arteriole to the capillary bed. A further increase in total cross-sectional area of 20% and 33% occurred in the first and second order of postcapillary venules, respectively. The cross-sectional area then decreased in the third order of collecting vessels to 95% of that in the capillary bed. The mean diameter and range of the first and second order venous vessels were 10μ (8-12μ) and 26μ (10-33μ), respectively. Approximately 75% of the blood was in the collecting vessels and only 3-10% in the capillary net. The endothelial surface area decreased from capillary through the first two orders of postcapillary venous vessels in the ratio of 6:4.7:1. (Aided by the Dept. of the Army,OSG., and the Nat'l Heart Institute, PHS.)

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